Identification of Bacterial Efflux Pump Inhibitors

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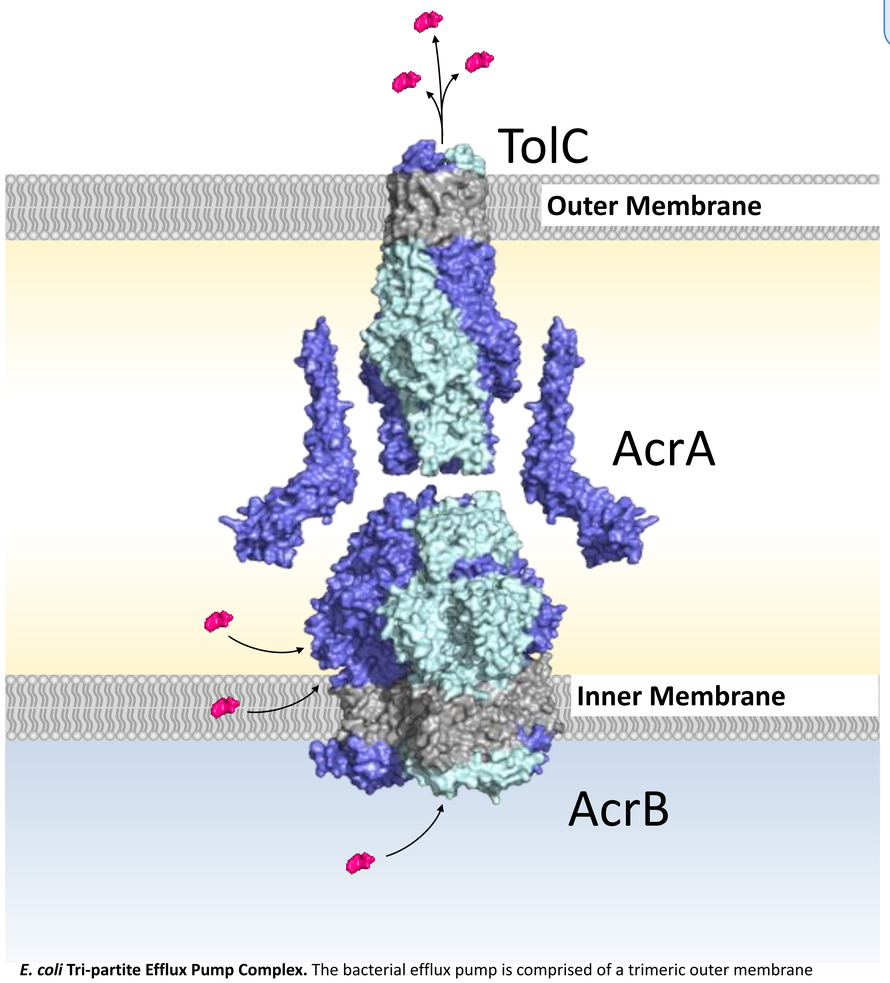
Introduction

Bacteria such as *Escherichia coli* contain efflux pumps (EP) that allow the cell to expel antibiotics into the extracellular environment giving them the ability to be multi-drug resistant. The tri-partite complex includes components AcrA, AcrB, and TolC. Together, they make up the efflux pump which is activated by the proton motive force. This phenomenon has been an increasing threat to modern medicine limiting the number of antibiotics available to treat disease. To address this issue that these efflux pumps confer on a bacterial cell, we screened a large number of small molecules with the best potential to bind to the tripartite pump complex and inhibit normal efflux function. Our hypothesis is that by identifying strong binding compounds to the efflux complex, these would block small molecule efflux and restore the bacteria's sensitivity to them.



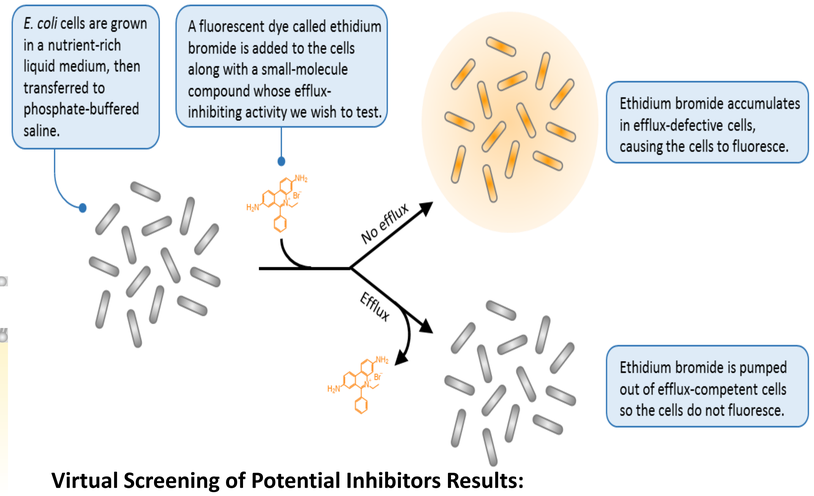
***E. coli* Tri-partite Efflux Pump Complex.** The bacterial efflux pump is comprised of a trimeric outer membrane spanning region (TolC), an inner membrane transporter (AcrB), and a periplasmic adaptor protein (AcrA). To drive antibiotics into the environment, AcrB uses energy gained from the proton motive force to move antibiotics across the TolC channel.

Methods

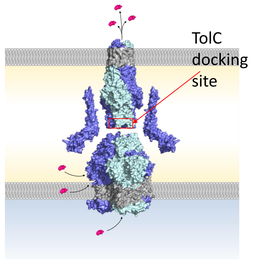
Identifying potential efflux pump inhibitors:

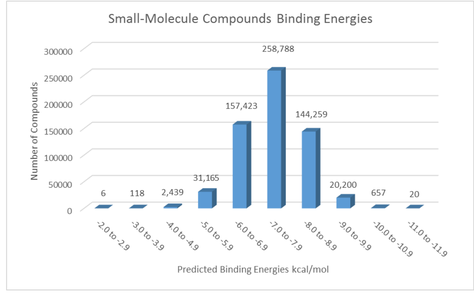
1. Obtain high-quality resolution images of efflux pump components from protein databank found in http://www.rcsh.org
2. Identify small molecule compounds that bind to TolC docking site at http://zinc.docking.org/
3. Screen compounds by docking them to TolC using the following program: http://vina.scripps.edu/
4. Rank compounds in order of decreasing binding energies (based on intermolecular attractions and forces). Highly negative binding energies indicate favorable binding to pump.
5. Obtain promising compounds from commercial sources and run *in vivo* efflux assays

Measuring efflux activity *in vivo:*



Virtual Screening of Potential Inhibitors Results:



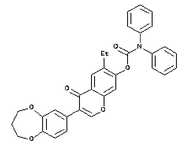


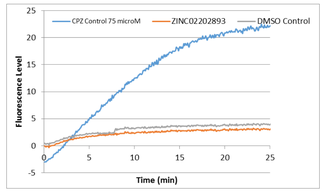
- We screened 614,575 compounds from the ZINC database that had the ability to dock to the highly conserved TolC complex.
- From these, 10 compounds were chosen based on:

1. Low/favorable binding energies $\leq -10.2 \frac{\text{kcal}}{\text{mol}}$
2. Compound availability in commercial distributers

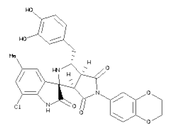
Results

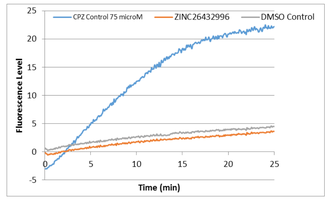
ZINC02202893





ZINC70705362





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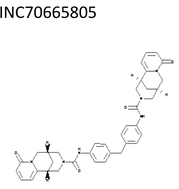

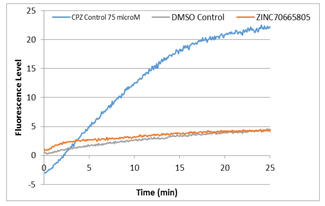

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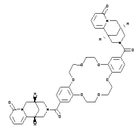

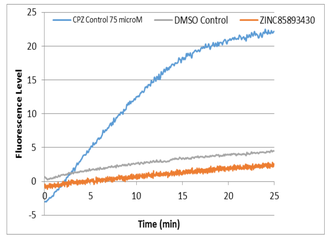

Conclusion

After virtually screening 614,575 small-molecule compounds we chose nine that proved to have the best potential to inhibit efflux activity based on binding energies. Accumulation assay trials were conducted and all of the nine compounds failed to inhibit EthBr efflux out of the bacterial cell. This is represented by the lack of high accumulating fluorescent levels. This enables us to determine that the TolC binding site is not an appropriate target for potential inhibitors to bind as it does not significantly affect efflux activity. In the near future we will work on identifying alternative binding sites along the tri-partite complex.

Acknowledgements:

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